

The Effect of Amino Nitrogen on the Energetics of Ruminal Bacteria and Its Impact on Energy Spilling

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Introduction

Ruminant feeding systems have generally been based on the assumption that energy should be given first consideration, but there is increasing evidence that amino acid supply can in many cases limit the production of high producing ruminants. Recent work has stressed the importance of 'escape' or 'bypass' protein in meeting amino acid requirements, but microbial protein is generally the dominant source of amino acids passing into the small intestine. The 1989 NRC recommendations for dairy cattle used a static equation to predict microbial protein flow from the rumen and assumed that ruminal microorganisms always had the same growth efficiency.

Most bacteria can utilize ammonia as a nitrogen source, but many bacteria prefer preformed amino acids. The impact of preformed amino acids on the growth efficiency of ruminal bacteria has not been clear-cut. In vitro batch cultures of mixed ruminal bacteria grew 60 to 400% more efficiently when amino acids or peptides, respectively, were provided, but the responses of carbohydrate-limited cultures were much less or even negligible. Some workers noted a 30% increase in microbial flow from the rumen when casein was added to a urea-based diet, but ruminally degraded protein has, in many cases, provided no benefit.

Materials and Methods

Ruminal contents were squeezed through cheesecloth. After gas production had buoyed small feed particles to the top and protozoa had settled to the bottom, bacteria were anaerobically transferred from the center of the flask to a Hungate tube. The ruminal fluid was diluted 10^8 -fold in basal media containing salts, cysteine, sulfide vitamins, minerals, volatile fatty acids, protein hydrolysate, soluble starch, cellobiose, sucrose, xylose, arabinose, and pectin. These predominant ruminal bacteria were grown in batch and continuous culture. Optical density was measured at 600 nm (18 mm tubes). Carbohydrate disappearance from cell-free

supernatants was measured by the anthrone method. Fermentation acids were determined by high pressure liquid chromatography. Cell protein was measured by the folin method. Cellular polysaccharide was determined by the anthrone method.

Results and Discussion

Predominant ruminal bacteria (PRB) that were obtained from a 10^8 dilution of ruminal fluid could be maintained as a mixed population for long periods of time, so long as they were provided with a complex mixture of carbohydrates. Carbohydrate-limited, ammonia-excess continuous cultures (.07/h) of PRB had a low maintenance energy requirement, but the non-growth energy dissipation of ammonia-limited, carbohydrate-excess PRB was approximately 10-fold higher (.96 versus .09 mg of hexose equivalent per mg of protein per h, respectively). Mathematical derivations indicated that this additional non-growth energy dissipation could be accommodated by a growth-rate independent energy spilling function. Peptides and amino acids had little impact on the yield of carbohydrate-limited, ammonia-excess continuous cultures (.07/h), but amino N caused a large increase in the growth rate and yield of energy-excess batch cultures. The amino N-dependent change in growth rate and yield indicated that the energy-excess batch cultures had the same capacity to spill energy as ammonia-limited, carbohydrate-excess PRB (.80 versus .86 mg of hexose equivalent per mg of protein per h, respectively). When the energy-excess batch cultures were provided with amino N, the growth rate increased, the difference between anabolic and catabolic rates was smaller, and less energy was spilled.

In the derivation of Pirt, the yield (Y) of a bacterium can be predicted from the maintenance energy coefficient (m), the theoretical maximum growth yield of the bacterium (Y_G), and the growth rate (μ):

$$1/Y = m/\mu + 1/Y_G.$$

Maintenance energy coefficients are typically measured under carbohydrate-limiting conditions, but carbohydrate-sufficient cultures often have a much inflated m . The variation in m was a lively subject of debate in the 1970's

, and the terms “overflow metabolism,” “slip reactions,” “uncoupling,” and “energy spilling” were all used as hypothetical explanations of the unexplained energy dissipation. Based on the observation that the apparent m of ammonia-limited cultures was higher, it was possible to modify the basic yield equation,

$$1/Y = m/\mu + 1/Y_G,$$

to include an energy spilling function (e):

$$1/Y = m/\mu + e/\mu + 1/Y_G,$$

where e is defined as the slope of the carbohydrate-excess Pirt plot minus the slope of energy-limited cells (m).

The Cornell Net Carbohydrate and Protein System (CNCPS) uses m and Y_G to predict the Y of ruminal bacteria. All ruminal bacteria are assigned the same Y_G , but the m of nonstructural carbohydrate-fermenting (NSC) bacteria is 3-fold greater than the m of

structural carbohydrate-fermenting (SC) bacteria (.3 and .1 mg of carbohydrate per mg of protein per h, respectively). In this regard, the yield of NSC bacteria is discounted by a higher m , but these bacteria are stimulated by amino N availability. The CNCPS predicts μ from the first order rate of carbohydrate degradation (K_d),

$$\mu = K_d,$$

but the effect of N on μ is ignored. If N is limiting:

$$\mu < K_d.$$

The CNCPS adjusts the Y of NSC bacteria with an empirical peptide/amino acid stimulation function, but this function stimulates Y even at very low K_d . Because amino N only increased the Y of PRB when carbohydrate was in excess and the μ permitted by ammonia was less than K_d , the CNCPS may be over predicting the benefit of amino N when the rate of carbohydrate fermentation is low.

Conclusions

The modification of the CNCPS to include an energy spilling term may provide a basis for estimating the microbial growth even if total N is restricted.